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**Electronic Supplementary Material**

# Title: Mechanistic study of the differences in lactic acid bacteria resistance to freeze- or spray-drying and storage

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The datasets generated and/or analyzed during the current study are available in the Data INRAE repository (https://doi.org/10.57745/AAARUZ).

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**Table SM1.** Culturability results of protected *Lb. bulgaricus* CFL1 and *Lpb. plantarum* WCFS1 after production and after freezing at -80 °C.

| | log (UFC/mL) | | dtpH1.5 (min) | |
|---|---|---|---|---|
| | After production | Frozen | After production | Frozen |
| CFL1 | 9.65[a] | 9.40[a] | 369[a] | 380[a] |
| IQR | 0.12 | 0.03 | 11 | 8 |
| WCFS1 | 11.17[b] | 11.52[b] | 358[a] | 346[a] |
| IQR | 0.06 | 0.45 | 8 | 10 |

Data presented as median and interquartile ranges (IQR). Different superscript letters within each method represent statistical differences between samples at a 95% confidence level.

**Table SM2.** Activation energy (Ea) determined from culturability loss rate according to the Arrhenius equation for the four studied samples, and values found in the literature for the inactivation of lactic acid bacteria.

| Bacterial strain | Drying method | Protectant | Water activity | Ea (kJ/mol) | References |
|---|---|---|---|---|---|
| *Lactobacillus delbrueckii* subsp. *bulgaricus* CFL1 | Spray-drying | Fructo-oligosaccharides and maltodextrin | 0.174 | 85 | This work |
| *Lactobacillus delbrueckii* subsp. *bulgaricus* CFL1 | Freeze-drying | Fructo-oligosaccharides and maltodextrin | 0.088 | 78 | This work |
| *Lactiplantibacillus plantarum* WCFS1 | Spray-drying | Fructo-oligosaccharides and maltodextrin | 0.141 | 84 | This work |
| *Lactiplantibacillus plantarum* WCFS1 | Freeze-drying | Fructo-oligosaccharides and maltodextrin | 0.033 | 47 | This work |
| *Lactococcus lactis* subsp. *lactis* | Freeze-drying | Sucrose | Not specified | 82 - 96 | Ziadi et al. (2005) |
| *Lactobacillus paracasei* ssp. *paracasei* | Freeze-drying | Trehalose | 0.11 – 0.22 | 63 - 71 | Aschenbrenner et al. (2012) |
| *Lactobacillus plantarum* CIDCA83114 | Spray-drying | Galacto-oligosaccharides and maltodextrin | 0.22 | 40 | Sosa et al. (2016) |

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**Table SM3**. Assignment of main infrared vibrational bands of the infrared spectra identified on the studied LAB in the 3060 to 975 $cm^{-1}$ region, according to literature.

| Wavenumber ($cm^{-1}$) | | Assignment | Main biomolecules associated (and corresponding cellular compound or compartment) | References |
|---|---|---|---|---|
| This work | Literature | | | |
| 1751 | ~1753<br>1750-1720<br>1741<br>1740-1730<br>1736 | νC=O | Esters from lipids<br>(Membrane lipids) | Girardeau et al. (2022); Le Gal et al. (1991); Meneghel et al. (2020); Naumann et al. (2006); Quilès et al. (2010); Saulou et al. (2013) |
| 1714 | 1716<br>1713<br>1715<br>1714<br>1713 | νC=O, νC=N, νC=C, δNH | Nucleic acids, esters, carboxylic acids, (DNA/RNA, nucleoid, ribosomes) | Girardeau et al. (2022); Guerrero et al. (2022); Meneghel et al. (2020); Naumann et al. (2006); Quilès et al. (2010) |
| 1548 | 1568-1531<br>1550-1563<br>1548<br>1545<br>1536<br>1530 | Amide II ((δN-H) + (νC-N)) | Proteins<br>(Membrane, cytoplasm) | Dianawati et al. (2012); Girardeau et al. (2022); Guerrero et al. (2022) Le Gal et al. (1991); Meneghel et al. (2020); Naumann et al. (2006); Quilès et al. (2010); Santos et al. (2015); Saulou et al. (2013) |
| 1515 | 1518<br>1516-1518<br>1515 | νCC, δCH | Proteins, aminoacids, tyrosine<br>(Membrane, cytoplasm) | Barth et al. (2007); Guerrero et al (2022); Le Gal et al. (1991); Naumann et al. (2006); Santos et al. (2015) |
| 1419 | 1415<br>1417 | δC-O-H | Carbohydrates, proteins, nucleic acids (DNA/RNA)<br>(Membrane, cytoplasm, nucleoid, ribosomes) | Barth et al. (2007); Guerrero et al. (2022); Le Gal et al. (1991); Meneghel et al. (2020); Santos et al. (2015) |
| | 1420-1405<br>1415<br>1404<br>1400 | νC-O sym of $COO^-$ | Lipids from phospholipids<br>(Membrane) | |
| 1400 | 1420-1405<br>1400 | νC=O sym of $COO^-$ | Proteins (amino acids), lipids (fatty acid), carbohydrates (peptidoglycan)<br>(Membrane, cytoplasm, cell wall) | Guerrero et al. (2022); Le Gal et al. (1991); Meneghel et al. (2020); |

| | | | | |
|---|---|---|---|---|
| | 1401<br>1400 | $\nu COO^-$ sym | Proteins<br>(Membrane, cytoplasm) | Naumann et al. (2006); Quilès et al. (2010) |
| 1221 | 1250-1220<br>1222-1200<br>1220 | $\nu P{=}O$ asym of $PO_2^-$ | Nucleic acids (phosphodiester of DNA/RNA), carbohydrates (teichoic and lipoteichoic acids, peptidoglycan) | Girardeau et al. (2022); Guerrero et al. (2022); Naumann et al. (2006); Quilès et al. (2010); Santos et al. (2015) |
| | 1220 | νC–O–C | Lipids of phospholipids, unsaturated fatty acids (Membrane, nucleoid, ribosomes, cell wall) | |
| 1156 | 1160–1253<br>1155<br>1153 | νC–O | Proteins, nucleic acids<br>(Membrane, cytoplasm) | Barth et al. (2007); Girardeau et al. (2022); Guerrero et al. (2022); Quilès et al. (2010); Saulou et al. (2013) |
| | 1155 | νC-O, νC-C,<br>C-O-C deformation | Polysaccharides, sugar rings (cell wall, peptidoglycan) | |
| 1058 | 1076-1052<br>1058 | νCO, νCC, δOCH, $\nu PO_2^-$, νC-OH, νC-O-C sym, νP-O-C sym | Carbohydrates (oligosaccharides, polysaccharides, sugar rings, pectin, peptidoglycan)<br>Nucleic acids (deoxyribose),<br>Lipids (phospholipid phosphate) (membrane, cytoplasm, nucleoid, cell wall) | Girardeau et al. (2022); Guerrero et al. (2022); Meneghel et al. (2020); Quilès et al. (2010) |

ν = stretching, δ = bending

**References Table SM3**


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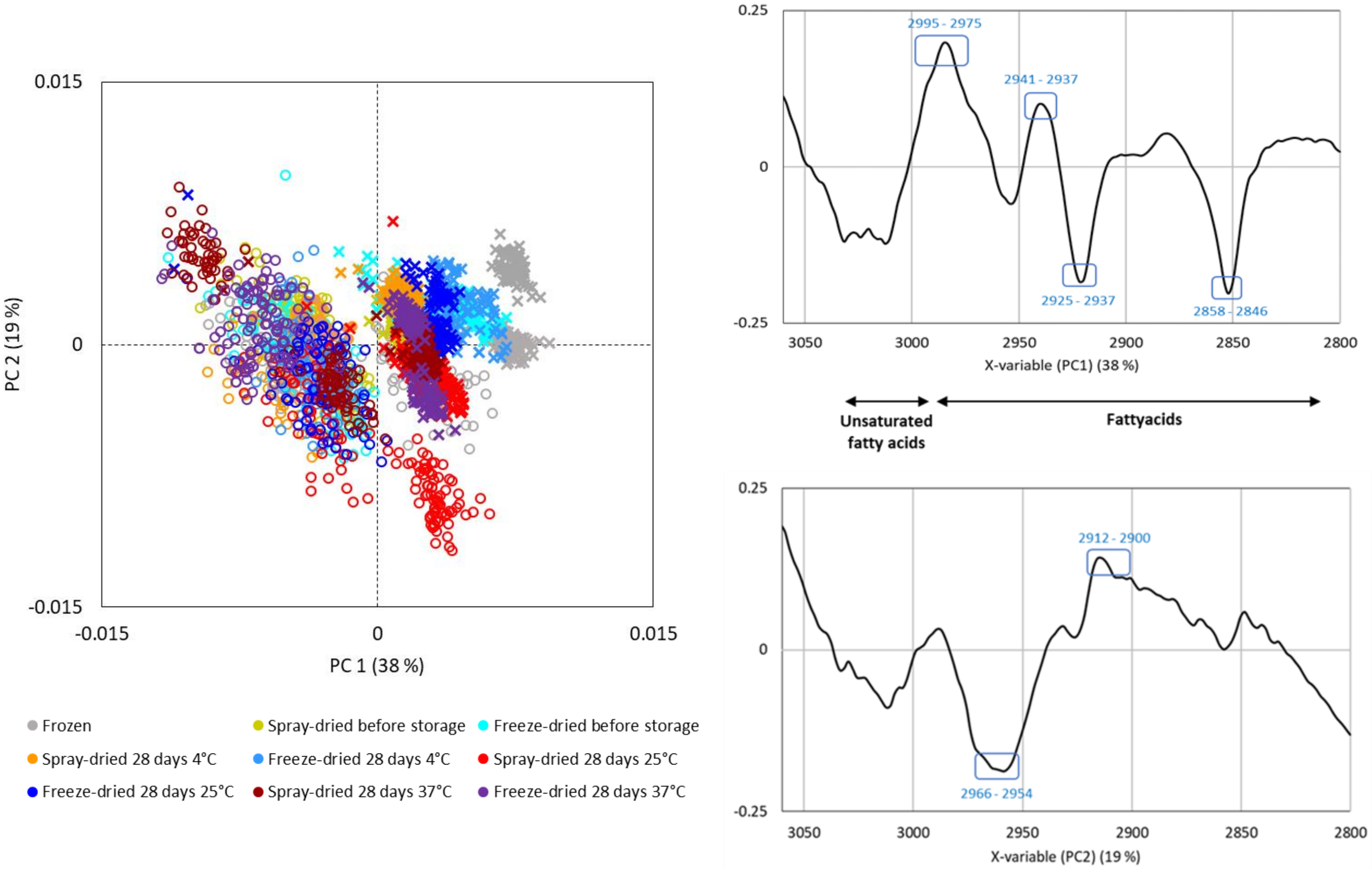


**Figure SM1.** Principal component analysis (PCA) of the FTIR normalized and corrected spectra of *Lb. bulgaricus* CFL1 (circles) and *Lpb. plantarum* WCFS1 (crosses) frozen, freeze-dried and spray-dried samples before storage and after storage for 28 days at 4, 23 and 37°C, in the spectral region 3060 - 2800 cm$^{-1}$. (A) score plot of PC1 vs. PC2; (B) and (C) loading plots for PC1 and PC2 respectively. Both biological replicates are plotted independently.

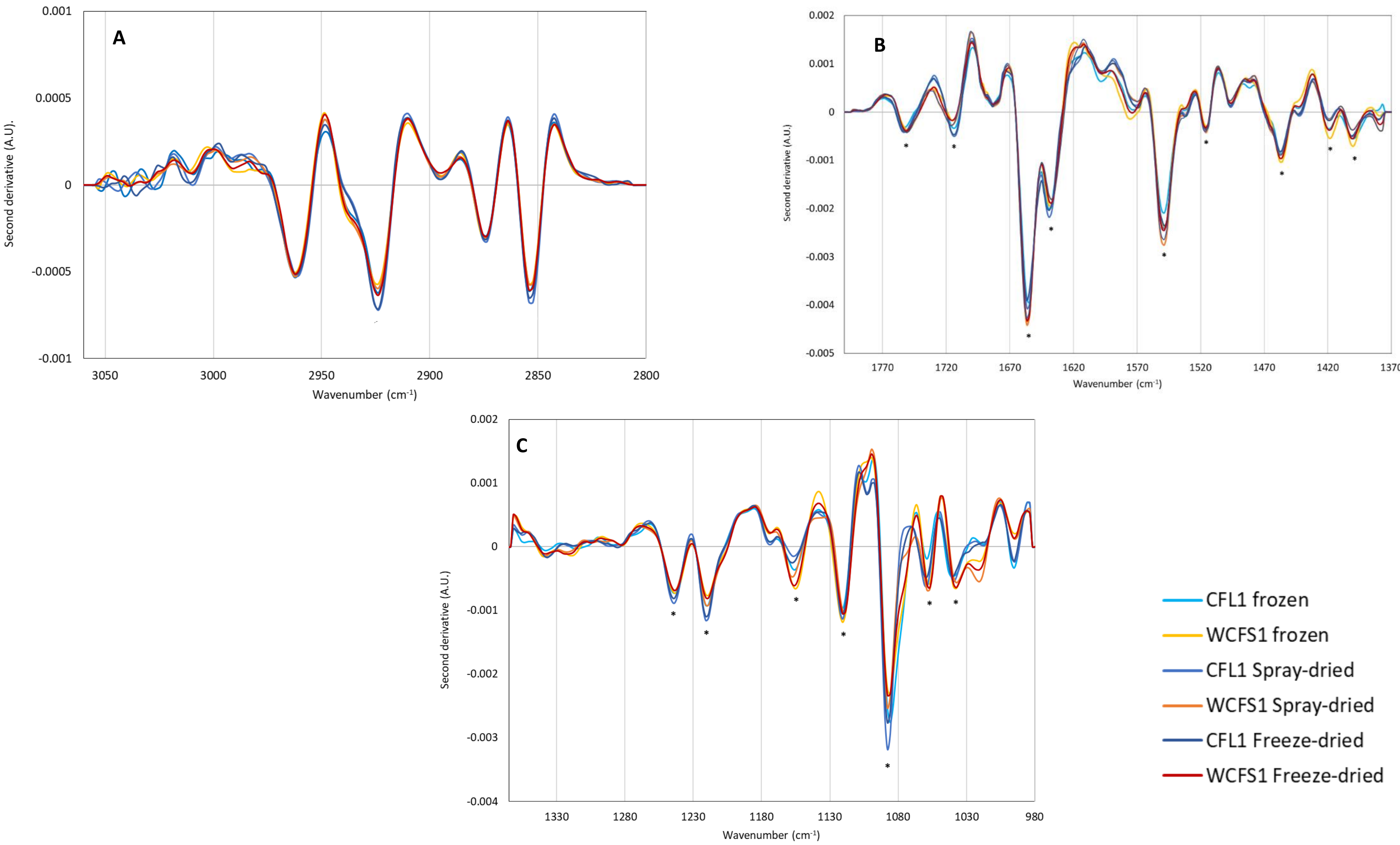


**Figure SM2.** Mean second derivatives of the normalized spectrum of the frozen, freeze-dried and spray-dried cells on the (A) 3060 – 2800 $cm^{-1}$, (B) 1800 – 1370 $cm^{-1}$ and (C) 1370 – 975 $cm^{-1}$ regions. The asterisks show the peaks evaluated in terms of peak height changes before and after drying.